

Metal Toxicity in the Central Nervous System

by Thomas W. Clarkson*

The nervous system is the principal target for a number of metals. Inorganic compounds of aluminum, arsenic, lead, lithium, manganese, mercury, and thallium are well known for their neurological and behavioral effects in humans. The alkyl derivatives of certain metals—lead, mercury and tin—are specially neurotoxic. Concern over human exposure and in some cases, outbreaks of poisoning, have stimulated research into the toxic action of these metals.

A number of interesting hypotheses have been proposed for the mechanism of lead toxicity on the nervous system. Lead is known to be a potent inhibitor of heme synthesis. A reduction in heme-containing enzymes could compromise energy metabolism. Lead may affect brain function by interference with neurotransmitters such as γ -amino-isobutyric acid. There is mounting evidence that lead interferes with membrane transport and binding of calcium ions.

Methylmercury produces focal damage to specific areas in the adult brain. One hypothesis proposes that certain cells are susceptible because they cannot repair the initial damage to the protein synthesis machinery. The developing nervous system is especially susceptible to damage by methylmercury. It has been discovered that microtubules are destroyed by this form of mercury and this effect may explain the inhibition of cell division and cell migration, processes that occur only in the developmental stages. These and other hypotheses will stimulate considerable experimental challenges in the future.

Introduction

Metals are among the most ancient poisons known to man. Mercury, named quicksilver by Aristotle, was described by Pliny as an occupational scourge to miners in Spain. Only criminals and slaves were employed in the notorious Almaden mines (1). Lead exposure of the ruling classes has been identified as a major cause for the decline of the Roman empire (2).

The nervous system is the principal target for many toxic metals. Mercury and lead will be discussed in detail. Aluminum has been implicated in senile dementia in dialysis patients (3), arsenic is well known to produce peripheral neuropathies (4), manganese damages specific centers in the brain to produce symptoms remarkably similar to Parkinson's disease (5), thallium wreaks havoc on the nervous system by mimicking potassium (6), and lithium has such remarkable effects that it is used to this day in the treatment of chronic depression (7). The most severe damage is produced by organo-metallic forms such as methylmercury (8), tetramethyl lead (9), and tin (10).

Lead and Mercury Historical Background

Time does not permit a discussion of all these metals. Instead, lead and mercury have been selected for a brief

review. In the 1960s, lead was emerging into a major public health problem. Ingestion of paint chips in inner city homes had caused, by the 1950s, many cases of severe encephalopathy, often having a fatal outcome. The survivors suffered permanent brain damage (11).

Subsequently it was discovered that the exposure to lead was more widespread and pervasive. Lead-containing dust both within and outside the home was elevating children's blood lead to toxic levels. The source was not only leaded paint but industrial emissions. We began to suspect leaded gasoline. More subtle effects were detected in the central nervous system, including behavioral disorders and intelligence deficits (12).

The discharge of methylmercury into an ocean bay in Japan in the 1950s led to a mass health disaster (13). The bay gave a name to a new clinical syndrome—Minimata Disease. From the original 120 cases of severe brain damage, there are now identified thousands of follow-up cases. In the 1960s it was realized that methylmercury could be produced without the help of human industry. Jensen and Aine Jernelov (14) discovered that microorganisms in sediments of lakes and rivers could methylate inorganic mercury, starting a process of bioaccumulation in aquatic food chains leading to human intake from fish. The report of high concentrations of methylmercury in many species of edible fish in Canada and the U.S. led to a near panic reaction. Large areas of fresh water for sports and commercial fishing were shut down, and even some species of ocean fish were banned from interstate commerce (15).

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In such a situation where we knew little about human dose-response relationships or mechanisms of action, the National Institute of Environmental Health Sciences encouraged and supported a broad program of research into how these metals damaged the nervous system. This is a brief report of some of this research.

Studies on Mechanisms of Action

Like most other toxic metals, lead and mercury exist as cations, and as such, can react with most ligands present in living cells. These include such common ligands as SH, phosphate, amino, and carboxyl. Thus they have the potential to inhibit enzymes, disrupt cell membranes, damage structural proteins, and affect the genetic code in nucleic acids. The very ubiquity of potential targets presents a great challenge to investigations on mechanisms of action.

Investigations on metal action have followed developments in biology. Studies of heavy metal action on enzymes in the 1920s paralleled the current interest of biochemistry at that time period in metabolic pathways (16). Indeed, metals were found to be useful in the isolation and purification of enzymes. Studies on the mechanism of action of arsenicals in World War II ultimately lead to the discovery of α -lipoic acid, the essential cofactor of pyruvate dehydrogenase (17).

The 1950s saw dramatic developments in membrane transport work. This in turn lead to the discovery that certain transport processes were highly sensitive to certain metals. The uranyl ion was used by Rothstein as a powerful tool to elucidate the membrane binding sites involved in glucose transport (16). He proposed the idea of geographical selectivity of metals (18). Metals would first react with surface ligands on the plasma membrane. Some surface sites are involved in glucose transport. As the metal moved into the membrane itself, the permeability was increased. Once inside the membrane, a number of intracellular processes were affected.

The 1960s and 1970s saw developments in studies of intracellular particles, protein synthesis, nucleic acid metabolism, and most recently, calcium and the cytoskeleton. In the case of lead and methylmercury, these biological advances led to progress in our knowledge of the toxicology of these metals.

In discussing some ideas on the mechanism of action of lead and mercury, one must first admit that current knowledge of the functioning of the nervous system does not allow us to formulate a precise cause-effect synthesis of effects at the biochemical and cellular levels to explain the clinical signs and symptoms. The assumption is that some day this will be possible. In the meantime, basic mechanisms of action can still give insight into how cell function is affected and can give plausible explanations for many of the effects seen at the whole animal and epidemiological levels.

Lead—Mechanisms of Action

The effects of lead on the central nervous system in children range from acute encephalopathy to a chronic,

subtle change in behavior and cognition. Cerebrovascular damage is probably involved in acute high doses. The basic lesions underlying the chronic effects are a matter of great interest and research activity.

Several fascinating theories about the mechanism of lead-induced damage have been proposed. Lead is well known to disrupt heme synthesis (11). The loss of heme-containing enzymes should affect mitochondrial function (19), producing adverse effects on energy metabolism. The cells of the blood-brain barrier are especially rich in mitochondria, making the blood-brain barrier susceptible to lead poisoning (20).

Lead may affect neurotransmission by interference with neurotransmitters (21). In fact, aminolevulinic acid (ALA), produced as a result of lead action on heme synthesis, has a similar chemical structure to the neurotransmitter γ -amino-isobutyric acid (GABA). Action on the GABA system might produce signs of neurotoxicity similar to those seen in severe lead poisoning (22). ALA may bind to GABA receptors (23). Action of lead is also a possibility on acetylcholine and catecholamine systems.

Some of these effects may be secondary to a more primary point of attack by lead. In this respect there is now great interest in the effects of lead on calcium metabolism and transport (24). Kostial and Vouk (25) were among the first to demonstrate the importance of lead-calcium interactions (Fig. 1). Using the perfused cervical ganglion of the cat, they demonstrated that lead can inhibit the release of acetylcholine. The effect persisted on washing with lead-free Locke solution but was dramatically reversed when the concentration of calcium was raised to five times the normal level. These

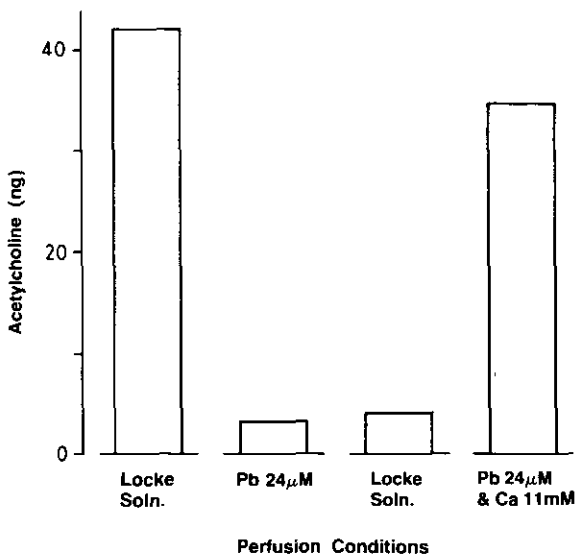


FIGURE 1. The antagonism of calcium on the effects of lead on acetylcholine release from the cat cervical ganglion *in vitro*. Lead inhibits acetylcholine release (second column). The effect persists when the preparation is washed with lead-free Lockes solution (column 3), but is dramatically reversed when the calcium concentration is increased. From Kostial and Vouk (25).

effects could be produced with lead concentrations as low as those expected to be present in brains of lead poisoned animals.

Since the publication of this report, considerable advances have been made in our knowledge of the role of calcium in the nervous system (Fig. 2). Calcium enters the presynaptic terminal via the calcium channel in response to the arrival of a wave of depolarization (24). Inside the cell, calcium activates calmodulin, leading to the fusion of acetylcholine vesicles with the plasma membrane and the release of acetylcholine. There is now mounting evidence that lead competitively inhibits calcium entry. The findings of Kostial and Vouk can be explained by the action of lead on this channel. More recently, Cooper and associates at Cincinnati (26) have elegantly demonstrated this competition. They measured the magnitude of the endplate potential that results from calcium entry into the cell and were able to show competitive inhibition by lead and calculate a Lineweaver-Burke inhibitory constant in the micromolar range.

Atchison and Narahashi (27) have provided evidence that lead can produce intracellular effects. Miniature endplate potentials (MEPP) are produced by the spontaneous and continuous release of packages of acetylcholine into the synaptic cleft. In the presence of lead, MEPPs are increased. This probably results from entry of lead via the calcium channel and interference with those processes regulating intracellular calcium such as mitochondrial uptake.

Indeed, mitochondrial uptake of lead has been elegantly demonstrated by Silbergeld and Goldstein in electron probe measurements in endothelial cells of brain capillaries (28). Action of lead on mitochondrial respiration may underlie the damage that lead is known to produce to the blood-brain barrier. Goyer first proposed a mitochondrial effect of lead to explain damage to kidney cells that, like the endothelial cells of the

blood-brain barrier, require a copious supply of ATP (11).

A discussion of the mechanism of lead toxicity would be incomplete without mention of what appears to be an important cellular protective process. In studies on the renal deposition of lead, Goyer et al. (29) had noted the appearance of electron dense inclusion bodies in the cell nucleus. These inclusion bodies contained high concentrations of lead and an acidic protein (30). Protein synthesis was required for their formation (31). According to McLachlin et al. (31), lead combines first with soluble proteins in the cytosol, followed by aggregation of the lead-protein complex to form visible inclusion bodies. The latter are transported into and accumulated in the cell nucleus. The formation of inclusion bodies protects the cell, and particularly the mitochondria, from the toxic effects of lead (11,32).

More recently, Holtzman et al. (33) have shown that intranuclear inclusion bodies in astrocytes protect brain cells from mitochondrial damage. Indeed, the susceptibility of the very young animal may be due to the fact that intranuclear bodies cannot be induced at an early age. In line with Holtzman's ideas, Egle and Skelton (34) found that the protein associated with these bodies was not detectable in younger animals but its presence in tissues increased dramatically with age.

Mercury—Mechanisms of Action

The onset of clinical signs and symptoms is shown in Figure 3 in a victim of the outbreak in Iraq in 1972. Paresthesia is usually first to appear. Later this is followed by more serious effects such as ataxia and other signs of loss of coordination such as slurred speech. Constriction of the visual field frequently occurs as a late sequelae. All these signs and symptoms arise from damage to the nervous system, particularly the central nervous system. In fact, damage is remarkably selective, being limited to specific focal areas such as the granule cells of the cerebellum and the neurons in the interstices of the visual cortex (35).

Such selective action is surprising in view of the chemical properties of methylmercury. The mercury cation reacts rapidly and with high affinity to SH groups (36). When present at sufficient concentrations, it will inhibit any SH-containing enzyme. Furthermore, it readily distributes to all tissues in the body, and the brain has concentrations no higher than other tissues (37).

The first clue to its selective action was the discovery that methylmercury inhibited protein synthesis in the brain (38). Protein synthesis was affected in the latent period well before clinical signs appeared. Syversen studied this effect in three different neuronal cell types (39). Rats were treated with a low dose of methylmercury and sacrificed at various times. Cells were harvested from different parts of the brain and tested for protein synthesis. Protein synthesis was inhibited in all three cell types. In the two nontarget cells (neurons from the cerebrum and Purkinje cells that are usually spared in methylmercury poisoning) recovery took

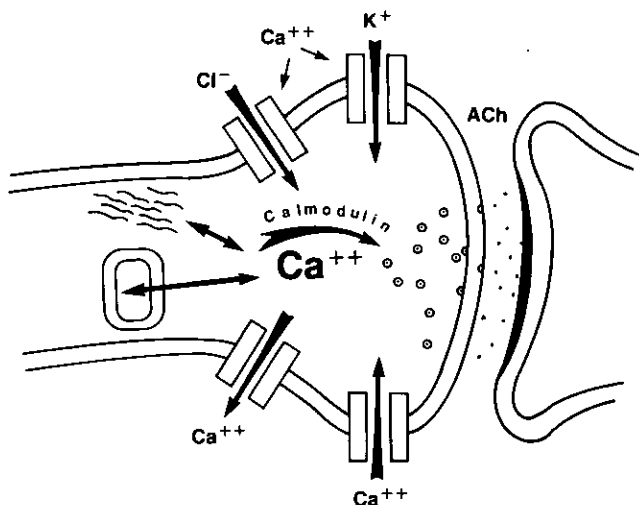


FIGURE 2. A schematic representation of the role of calcium in the presynaptic nerve terminal. For further details, see text.

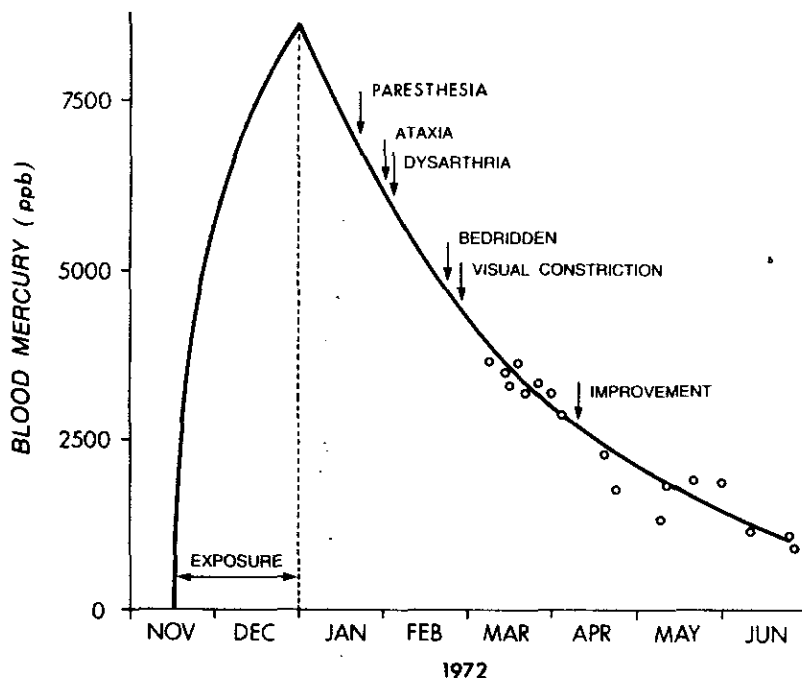


FIGURE 3. The onset of clinical signs of symptoms in a victim of methylmercury poisoning in the Iraq outbreak of 1971-72. The solid line represents recapitulated blood levels based on data on intake and the pharmacokinetics of methylmercury. (○) Actual observed blood concentrations.

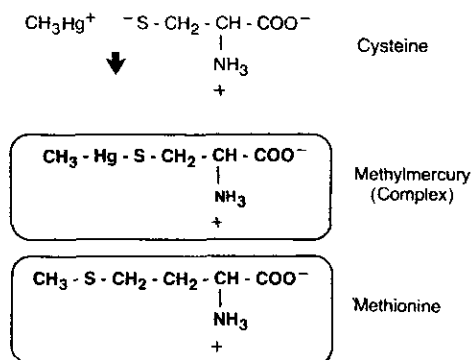


FIGURE 4. A comparison of the structure of methionine with the methylmercury complexes with cysteine and homocysteine.

place. In the granule cell, which is a target cell (usually damaged in methylmercury poisoning), no recovery took place. Thus, selective action on the brain may be due to the fact that certain cells are susceptible because they cannot repair damage from methylmercury. The initial damage is nonselective, consistent with the chemical properties of methylmercury.

Verity and his colleagues (40) have identified the step in protein synthesis most sensitive to methylmercury. The peptide elongation can be affected at high levels of methylmercury, but the first stage of synthesis associated with transfer RNA may be the most sensitive. There is no selective inhibition of formation of any spe-

cial proteins or group of proteins. Cheung and Verity (40) suggested that methylmercury was inhibiting one or more of the amino acyl tRNA synthetase enzymes.

The first amino acid to start the polypeptide chain is always methionine. Perhaps it may be a coincidence that methylmercury may enter the brain as an amino acid complex structurally similar to methionine. It had been known for some time that certain thiol-containing amino acids accelerate transport across the blood-brain barrier and that large neutral amino acids can inhibit entry into the brain (41-43). The process appears to be stereospecific, as the D, but not L, enantiomorph of the cysteine methylmercury complex enters the brain (43). Thus, Hiriyama suggested that "the blood-brain transport system (for amino acids)" participates in some way in the penetration of methylmercury into the brain (43). Recently, Aschner and Clarkson (44) suggested that transport of methylmercury as the cysteine complex may be due to its structural similarity to methionine (Fig. 4). Methionine shares with the other large neutral amino acids a common carrier in the membranes of the endothelial cells of the brain capillaries (45,46). Consistent with this suggestion, Aschner and Clarkson report that methionine also inhibited the entry of the cysteine complex into the brain. Thus the possibility arises that methylmercury not only enters the brain as a complex structurally similar to methionine but may exist in this form until it penetrates to the ribosomes and the site of initiation of protein synthesis.

As in the case of lead, the developing nervous system

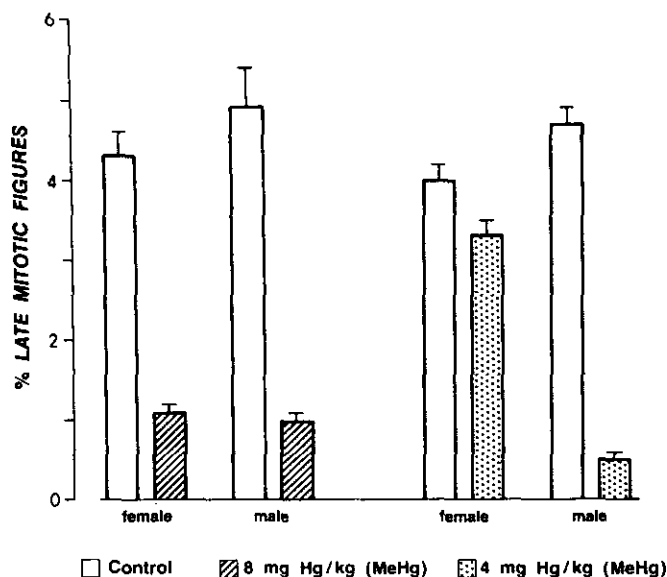


FIGURE 5. The effects of methylmercury on cell division in the developing brain. Two-day-old mice were given 4 or 8 mg Hg/kg as methylmercury chloride per os and sacrificed 24 hr later. The total number of mitotic figures in the external granule layer of the cerebellar cortex were recorded in matched sections and classified as early or late. Data are plotted as the percentage of late mitotic figures (anaphase and telophase). Bars are the standard errors ($n = 10-15$). Adapted from (54).

is the most susceptible stage of the life cycle to methylmercury (47,48). Evidence from human autopsies indicate derangement of brain structure and smaller brain size without selective damage as seen in the adult (49). One reason for this higher susceptibility is that mercury affects processes unique to the developing nervous system, namely cell migration and cell division.

Once more, advances in basic biology, in this case the structure of the cytoskeleton, has led to insight into molecular mechanisms. Methylmercury leads to rapid depolymerization of microtubules (50). Cells exposed to low concentrations rapidly lost microtubule structures (51,52). Two protein monomers, α and β tubulin, aggregate to form microtubules. Formation of the tubule takes place at one end of the forming tubule, whereas the tubule dissociates at the other end (53). Methylmercury reacts with the SH groups on tubulin monomers to disrupt the assembly process. The dissociation process continues, thus leading to depolymerization of the tubule.

As microtubules are essential for cell division (they are the main component of the mitotic spindle), effects on cell division can be understood. Effects on the division of granule cells in the developing cerebellum of the mouse are illustrated in Figure 5. A single dose of 8 mg/kg to 2-day-old mice inhibits cell division in both sexes, whereas the 4 mg/kg dose affects cell division only in male animals. It is of interest that this sex difference confirms findings in recent epidemiological studies in Canada (55). Furthermore, the 4 mg/kg dose pro-

duces the lowest brain levels of methylmercury at which any effects have been seen.

Conclusion

In conclusion, our knowledge of the mechanisms of action of lead and mercury develops in parallel with, and in most cases as a result of, advances in basic biology. Basic biology has now realized an exciting phase where specific hypotheses have been advanced at a molecular level. These hypotheses should lead to considerable experimental challenge in the future.

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